Journal of Computational Neuroscience 9, 259–270, 2000

Nonlinear Thermodynamic Models of Voltage-Dependent Currents

ALAIN DESTEXHE
Department of Physiology, Laval University, Quebec, Canada G1K 7P4 and Unité de Neurosciences Intégratives et Computationnelles, CNRS, UPR-2191, Avenue de la Terrasse, 91198 Gif-sur-Yuette, France
Destexhe@iaf.cnrs-gif.fr

JOHN R. HUGUENARD
Department of Neurology and Neurological Sciences, Stanford University, Stanford, CA 94305, USA

Received November 10, 1999; Revised February 17, 2000; Accepted

Action Editor:

Abstract. Hodgkin and Huxley provided the first quantitative description of voltage-dependent currents and adjusted their model to experimental data using empirical functions of voltage. A physically plausible formalism was proposed later by assuming that transition rates depend exponentially on a free-energy barrier, by analogy with the theory of reaction rates. It was also assumed that the free energy depends linearly on voltage. This thermodynamic formalism can accurately describe many processes, but the resulting time constants can be arbitrarily fast, which may also lead to aberrant behavior. We considered here a physically plausible solution to this problem by including nonlinear effects of the electrical field on the free energy. We show that including effects such as mechanical constraints, inherent to the structure of the ion channel protein, leads to more accurate thermodynamic models. These models can account for voltage-dependent transitions that are rate-limited in a given voltage range, without invoking additional states. We illustrate their applicability to fit experimental data by considering the case of the T-type calcium current in thalamic neurons.

Keywords: ion channels, gating, Hodgkin-Huxley models, Markov models, T-type calcium currents

1. Introduction

Hodgkin and Huxley (1952) provided the first quantitative characterization of the voltage dependence of ionic currents and its role in generating action potentials. The formalism they used invoked the assembly of several gating particles acting independently in a voltage-dependent manner. The voltage dependence of the rate constants were fit to voltage-clamp measurements using empirical functions of voltage, which conferred to the model a behavior consistent with action potential genesis in squid giant axon.

Instead of using empirical functions, it is also possible to deduce the functional form of the voltage dependence of the rate constants from thermodynamics.[1] These *thermodynamic models* (Eyring et al., 1949; Tsien and Noble, 1969; Hill and Chen, 1972; Stevens, 1978; Hille, 1992) provide a firm physical basis to constrain and parameterize the voltage dependence of rate constants, which are then used to fit voltage-clamp data. Thermodynamic models usually assume that a free-energy barrier is associated to the transition, by analogy with the theory of reaction rates (Eyring, 1935; Johnson et al., 1974). They also assume that the effect of the electrical field is linear on the free energy, which can be interpreted physically as reducing the conformational change to the translation of a freely moving electric charge or the rotation of rigid dipoles (Andersen and Koeppe, 1992; Hille, 1992; Johnston and Wu, 1995).

Although this approach has proved to be accurate for many purposes (reviewed in Hille, 1992), it has the major complication that the time constant can reach arbitrarily small values, which may lead to aberrant behavior. In reality, the complex molecular structure of ion channels imposes bounds on their transition rates. Because exponential functions of voltage cannot account for rate-limited transitions, this problem is usually solved by introducing a minimal rate (Borg-Graham, 1991), forcing the rate constants to saturate (Willms et al., 1999), or using additional voltage-independent rate-limiting transitions. These procedures are, however, largely empirical.

In this article, we consider a physically plausible framework to solve this problem. We introduce a functional form for the voltage dependence of rate constants that takes into account more realistic effect of the electrical field on the ion channel protein. We then show that this functional form naturally accounts for rate-limiting effects, and we illustrate its applicability by modeling the voltage-dependence of T-type calcium currents.

2. Methods

Whole-cell voltage-clamp recordings of the T-type calcium current were obtained from thalamic relay neurons acutely dissociated from the ventrobasal thalamus of young rats (P8–P15). All voltage-clamp recordings were at a temperature of 24 degree centigrade. The methods were described in detail in Huguenard and Prince (1992).

The voltage-clamp behavior of the T-type Ca^{2+} current (I_T) was modeled using a Hodgkin and Huxley (1952) type model. Due to the nonlinear behavior of Ca^{2+} currents, I_T was represented using the constant-field equations (see Hille, 1992):

$$I_T = \bar{P}_{Ca}\, m^2 h \frac{Z^2F^2V}{RT} \frac{Ca_i - Ca_o \exp(-ZFV/RT)}{1 - \exp(-ZFV/RT)}, \tag{1}$$

where $\bar{P}_{Ca}$ (in centimeter per second) is the maximum permeability of the membrane to Ca^{2+} ions, $Z = 2$ is the valence of Ca^{2+} ions, F is the Faraday constant, R is the gas constant, and T is the absolute temperature in degrees Kelvin. Ca_i and Ca_o are the intracellular and extracellular Ca^{2+} concentrations (in M), respectively. The gating of I_T was represented by two inactivation gates (m) and one inactivation gate (h). m and h are the fraction of each gate in the open configuration. The m^2h formalism in Eq. (1) was deduced by maximum likelihood criteria from voltage-clamp experiments (Huguenard and Prince, 1992). These variables were described by the following first-order equations:

$$\frac{dm}{dt} = \alpha_m(V)(1-m) - \beta_m(V)m \tag{2}$$

$$\frac{dh}{dt} = \alpha_h(V)(1-h) - \beta_h(V)h, \tag{3}$$

where α_m and β_m are, respectively, the forward and backward rate constants for activation. For the inactivation gate, these constants are α_h and β_h, respectively.

The quantities directly observable using voltage-clamp experiments are the steady-state activation (m_∞), the activation time constant (τ_m), the steady-state inactivation (h_∞), and the inactivation time constant (τ_h). These quantities are given, respectively, by

$$m_\infty = \alpha_m/[\alpha_m + \beta_m] \tag{4}$$

$$\tau_m = 1/[\alpha_m + \beta_m] \tag{5}$$

$$h_\infty = \alpha_h/[\alpha_h + \beta_h] \tag{6}$$

$$\tau_h = 1/[\alpha_h + \beta_h]. \tag{7}$$

These equations were applied to model the T-type calcium current in thalamic neurons (see Section 3, Results), using different methods to determine the exact functional form of the rate constants α_m, β_m, α_h, and β_h. One of these methods consisted of an empirical fit to the experimental data (Huguenard and McCormick, 1992). The steady-state relations were fit using Boltzmann functions (Fig. 2A-B, solid lines), leading to the following optimal functions:

$$m_\infty(V) = 1/(1 + \exp[-(V+57)/6.2])$$

$$h_\infty(V) = 1/(1 + \exp[(V+81)/4]).$$

The voltage-dependence of time constants was represented by multiexponential functions that were fit to the experimental data. (Huguenard and McCormick, 1992), leading to the following expression for activation:

$$\tau_m(V) = 0.612 + 1/(\exp[-V+312)/16.7] + \exp[(V+16.8)/18.2]) \tag{8}$$

and for inactivation:

$$\begin{aligned} \tau_h(V) = 28 + \exp[-(V+22)/10.5] \\ \text{for } V \geq -81\text{mV} \\ \exp[(V+467)/66.6] \\ \text{for } V < -81\text{mV}. \end{aligned} \tag{9}$$

Here, two different functions were fit to the time constants τ_h obtained from inactivation protocols ($V \geq -81$ mV) or recovery from inactivation ($V < -81$ mV).

The T-current was simulated in current-clamp in a single-compartment neuron described by the following equation:

$$C_m \frac{dV}{dt} = -g_L(V - E_L) - I_T, \tag{10}$$

where $C_m = 0.88\,\mu\text{F/cm}^2$ is the membrane capacitance, $g_L = 0.038$ mS/cm^2 and $E_L = -77$ mV are the leak conductance and reversal potential, and I_T is the T-current as given by Eq. (1). These parameters were obtained by matching the model to thalamic neurons recorded *in vitro* (Destexhe et al., 1998).

All models were simulated using the NEURON simulation environment, which can solve cable equations and voltage-dependent conductances based on either differential equations or kinetic diagrams (Hines and Carnevale, 1997). Models were fit to experimental data using a simplex search procedure (Press et al., 1986). Fitting was performed simultaneously on two data sets: time constants and steady-state values (see Fig. 2). Each data set was given equal weight by renormalizing its mean-square error by its maximal amplitude. All computational models and fitting procedures were run on a Sparc 20 workstation (Sun Microsystems, Mountain View, CA).

3. Results

We first describe the empirical fitting of a Hodgkin-Huxley type model to the T-type calcium current in thalamic neurons. We then consider linear and nonlinear thermodynamic models and compare their adequacy to model the voltage-dependent properties of the T-current.

3.1. Hodgkin-Huxley Model of the T-Type Calcium Current

The T-type Ca^{2+} calcium current (also called low-threshold Ca^{2+} current) is responsible for the genesis of bursts of action potentials in many cell types, such as thalamic neurons (Jahnsen and Llinás, 1984). In thalamic neurons, this current was characterized by a number of voltage-clamp studies (reviewed in Huguenard, 1996). Several models of the T-current were proposed (reviewed in Destexhe and Sejnowski, 1997). To represent the "classical" Hodgkin-Huxley approach, which consists of adjusting rate constants to experimental data by using empirical functions of voltage (Hodgkin and Huxley, 1952), we have used here a model drawn by Huguenard and McCormick (1992), which is given in Section 2, Methods.

The T-type Ca^{2+} current is transient and has activation and inactivation characteristics similar to the fast Na^+ current but is slower, and its voltage range for activation and inactivation typically occurs around rest. Voltage-clamp recordings of the T-current are shown in Fig. 1A. A typical activation protocol for the T-current, consisting of a series of voltage steps from a hyperpolarized level (-100 mV) to various depolarized levels, reveals an inward current that activates and inactivates in a voltage-dependent manner (Fig. 1A1). A particular feature of the T-current is that the recovery from inactivation is very slow. This is shown using a voltage-clamp protocol in which the current is reactivated from a holding voltage where the current is fully inactivated (Fig. 1A2). The time constant of recovery, estimated from fitting an exponential to the peak currents (dashed line in Fig. 1A2), reveals time constants of about 300 ms, almost an order of magnitude slower than inactivation time constants.

The voltage-dependent properties of this current are illustrated in Fig. 2 (symbols). The steady-state relations and time constants obtained from voltage-clamp recordings of I_T are shown for several cells. Steady-state activation and inactivation had a sigmoidal voltage-dependence (Fig. 2A-B, symbols) consistent with the characteristics of the T-current in other types of neurons (Huguenard, 1996). Activation time constants showed a bell-shaped curve with values ranging from 1.5 to 20 ms and reached a constant value around 1.5 ms at depolarized levels (Fig. 2C, symbols). Inactivation time constants ranged from 25 ms to 120 ms (symbols > -81 mV in Fig. 2D), also showing a saturation to a

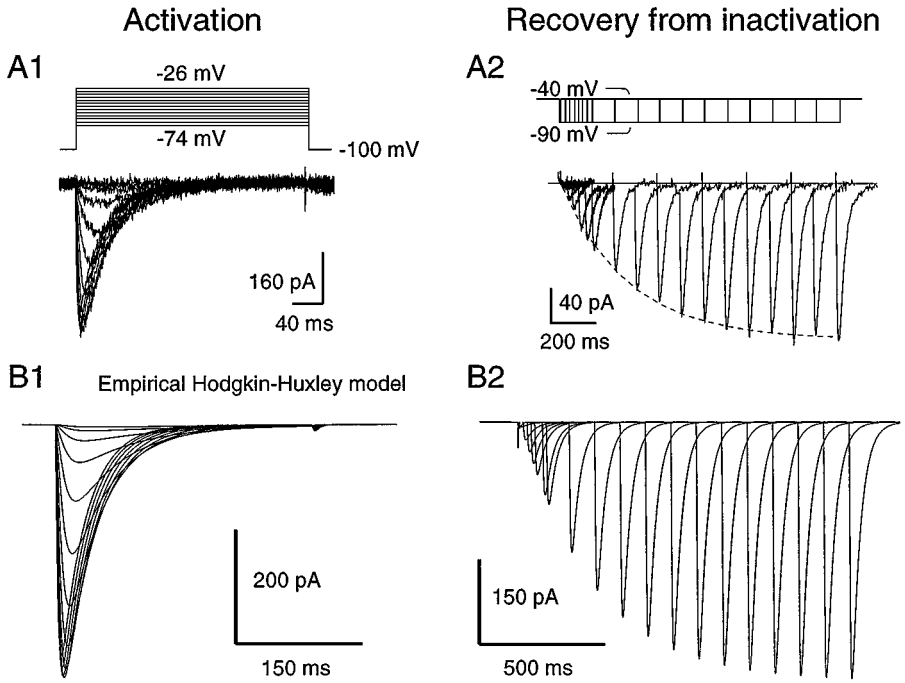

Figure 1. Experiments and models of the voltage-clamp behavior of the T-current in thalamic neurons. *Left panels*: Activation protocol. Command potentials at various levels were given after the cell was maintained at a hyperpolarized holding potential, leading to the activation of the current. *Right panels*: Protocol for the recovery from inactivation. From a holding voltage of −40 mV at which the current is fully inactivated, the current is stepped to −90 mV for variable durations, then stepped back to −40 mV. **A:** Experimental data from isolated thalamic neurons at 24°C. **B:** Empirical Hodgkin-Huxley model (see Section 2, Methods; the density of T-channels in B was $\bar{P}_{Ca} = 3 \times 10^{-6}$ cm/s).

constant value (about 25 ms) at depolarized levels and a slow recovery (symbols ≤ -81 mV in Fig. 2D).

Thus the T-current in thalamic relay neurons has activation and inactivation that are characterized by relatively slow time constants and a slow recovery from inactivation, almost an order of magnitude slower than inactivation.

The fit of a Hodgkin-Huxley model to these experimental data using empirical functions of voltage (see Section 2, Methods) is shown in Fig. 2 (solid lines). The behavior of this model in voltage clamp (Fig. 1B) accounted well for all voltage-clamp protocols with activation and recovery from inactivation shown in Fig. 1B1 and B2, respectively. However, in this model, τ_m and τ_h were fit using functions of voltage obtained empirically. Similar to the work of Hodgkin and Huxley (1952), this approach leads to a model that accounts well for the current-clamp behavior of the T-current in thalamic neurons (McCormick and Huguenard, 1992; see below).

3.2. Thermodynamic Models

Another possibility is to deduce the exact functional form of the voltage-dependence of the model from thermodynamics. This type of model assumes that the gating of ion channels operates through successive conformational changes of the ion channel protein. Consider an elementary transition between an initial (I) and a final (F) state, with a rate constant $r(V)$ that is voltage-dependent:

$$I \overset{r(V)}{\longrightarrow} F. \tag{11}$$

According to the theory of reaction rates (Eyring, 1935; Johnson et al., 1974), the rate of the transition depends exponentially on the free energy barrier between the two states:

$$r(V) = r_0\, e^{-\Delta G(V)/RT}, \tag{12}$$

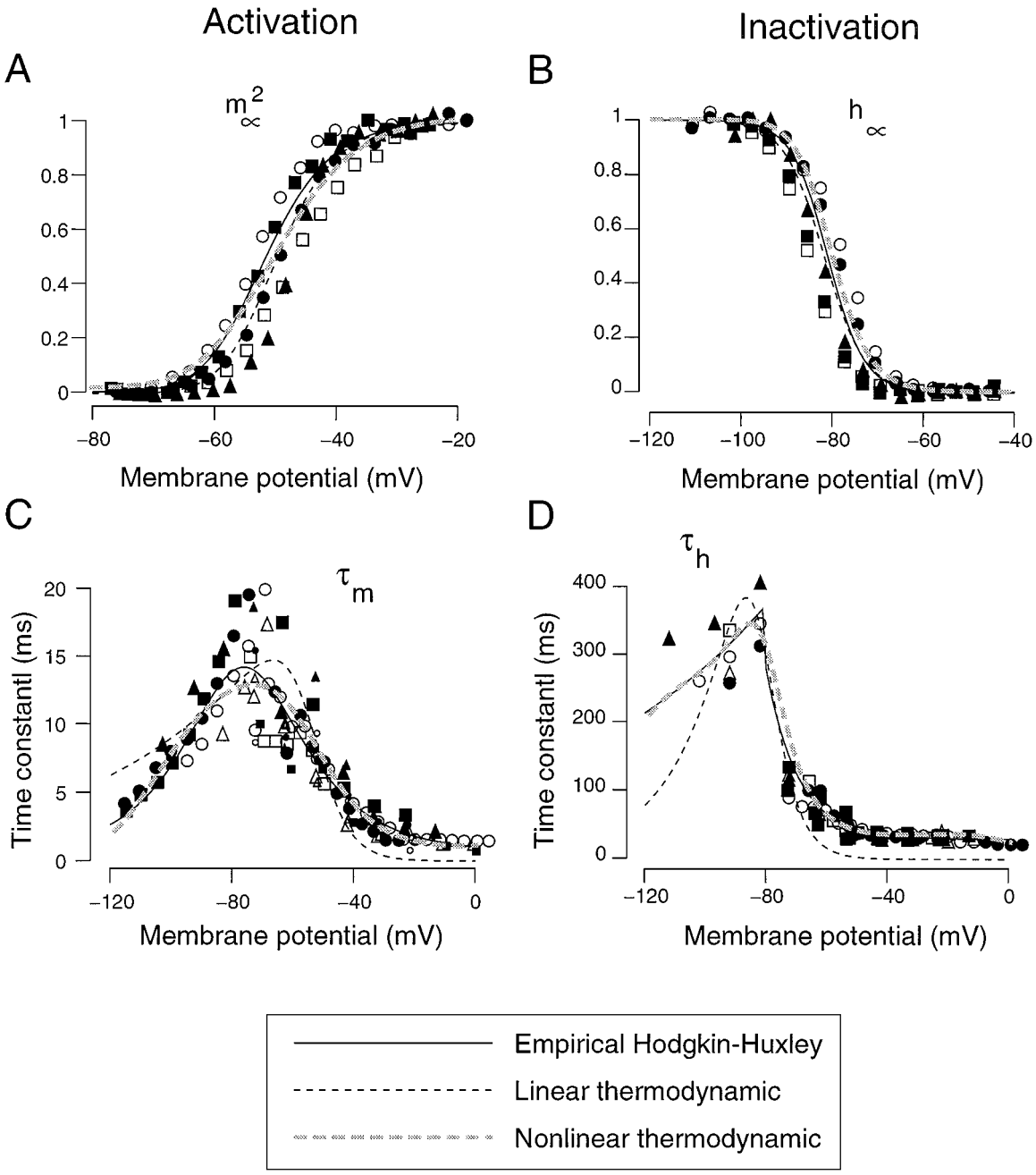

Figure 2. Fitting of differnt models to the T-current in thalamic relay neurons. In each panel, the symbols show the voltage-clamp data obtained in several thalamic neurons (from Huguenard and McCormick, 1992), and the continuous curves show the best fits obtained with three types of Hodgkin-Huxley models: an empirical model (solid lines), a linear thermodynamic model (thin dashed lines) and a nonlinear thermodynamic model (thick dashed lines). **A:** Steady-state activation (m^2_∞). **B:** Steady-state inactivation (h_∞). **C:** Activation time constant (τ_m). **D:** Inactivation time constant (τ_h). The leftmost symbols in D (≤ -81 mV) are the data from the slow recovery from inactivation of the T-current. See text for the values of the parameters.

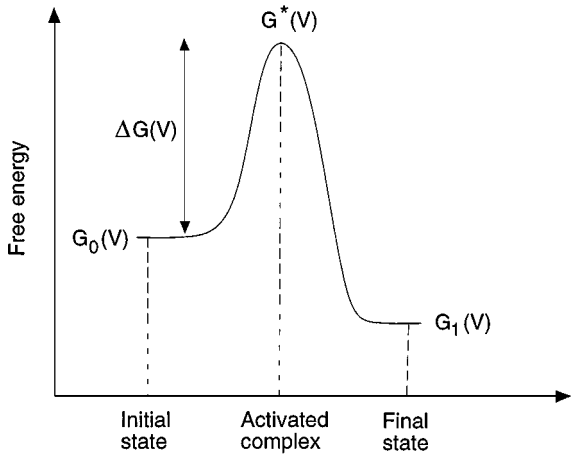

Figure 3. Schematic representation of the free energy profile of conformational changes in ion channels. The diagram represents the free energy of different states involved in a transition: the initial state, activated complex, and final state. The equilibrium distribution between initial and final states depends on the relative value of their free energy (G_0 and G_1). The rate of the transition will be governed by the *free energy barrier* ΔG, which is the free energy difference between the activated complex and the initial state.

where r_0 is a constant and $\Delta G(V)$ is the free energy barrier, which can be written as

$$\Delta G(V) = G^*(V) - G_0(V), \tag{13}$$

where $G^*(V)$ is the free energy of an intermediate state (activated complex) and $G_0(V)$ is the free energy of the initial state, as illustrated in Fig. 3. The relative values of the free energy of the initial and final states (G_0 and G_1) determine the equilibrium distribution between these states, while the kinetics of the transition depend on the size of the free-energy barrier $\Delta G(V)$.

Linear Thermodynamic Models It is usually assumed that a linear voltage dependence of the free energy is sufficient to describe the gating of ion channels (Tsien and Noble, 1969; Stevens, 1978; Borg-Graham, 1991; Andersen and Koeppe, 1992; Hille, 1992). According to these linear thermodynamic models, the free energy of a given state i can be written as

$$G_i(V) = A_i + B_i V, \tag{14}$$

where the constant A_i corresponds to the free energy that is independent of the electrical field, and the linear term $B_i V$ to the effect of the electrical field on isolated charges and rigid dipoles (Hill and Chen, 1972; Stevens, 1978; Andersen and Koeppe, 1992). For example, linear terms in V will result if the conformations differ in their net number of charges or if the conformational change is accompanied with the translation of a freely moving charge inside the structure of the channel (see Fig. 5A).

Applying Eqs. (12) to (14), the rate constant can be written as

$$\begin{aligned} r(V) &= r_0\, e^{-[(A^*+B^*V)-(A_0+B_0V)]/RT}, \\ &= r_0\, e^{-(a+bV)/RT}, \end{aligned} \tag{15}$$

where $a = A^* - A_0$ and $b = B^* - B_0$ represent differences between the constant and linear components of the free energy of the initial and activated states (according to Eq. (14)).

Consider the particular case of a reversible open-closed transition

$$C \underset{\beta(V)}{\overset{\alpha(V)}{\rightleftarrows}} O, \tag{16}$$

where C and O are, respectively, the closed and open states, and α and β are the forward and backward rate constants. Applying Eq. (15) to forward and backward reactions leads to the following expression for the voltage-dependence of rate constants:

$$\begin{aligned} \alpha(V) &= \alpha_0\, e^{-(a_1+b_1V)/RT} \\ \beta(V) &= \beta_0\, e^{-(a_2+b_2V)/RT}, \end{aligned} \tag{17}$$

where a_1, a_2, b_1, and b_2 are constants specific for this transition. In the following, this form with simple exponential voltage dependence of rate constants will be called *linear thermodynamic model*.

A further simplification is to consider that the conformational change consists of the movement of a freely moving gating particle with charge q (Hodgkin and Huxley, 1952; see also Borg-Graham, 1991). The forward and backward rate constants can be written as

$$\begin{aligned} \alpha(V) &= A\, e^{-\gamma qF(V-V_H)/RT} \\ \beta(V) &= A\, e^{(1-\gamma)qF(V-V_H)/RT}, \end{aligned} \tag{18}$$

where γ is the relative position of the energy barrier in the membrane (between 0 and 1), V_H is the half-activation voltage, and A is a fixed constant. This form was introduced by Borg-Graham (1991) to parameterize Hodgkin-Huxley models. Its parameters are very

convenient for fitting experimental data: V_H and q affect the steady-state activation and inactivation curves, whereas A and γ affect only the time constant with no effect on steady-state relations.

Linear Thermodynamic Model of the T-Current This formalism was used to constrain the fitting of experimental data by using rate constants dictated by Eq. (17). This procedure led to the following optimal expressions:

$$\alpha_m = 0.049 \exp[444\gamma_m(V + 54.6)/RT] \quad (19)$$

$$\beta_m = 0.049 \exp[-444(1 - \gamma_m)(V + 54.6)/RT] \quad (20)$$

$$\alpha_h = 0.00148 \exp[-559\gamma_h(V + 81.9)/RT] \quad (21)$$

$$\beta_m = 0.00148 \exp[559(1 - \gamma_h)(V + 81.9)/RT], \quad (22)$$

where $\gamma_m = 0.90$ and $\gamma_h = 0.25$. These expressions are drawn in Fig. 2 (thin dashed lines).

This model provided a good fit of the steady-state relations (Fig. 2A–B, thin dashed lines), but the fit to time constants was poor (Fig. 2C–D, thin dashed lines). In particular, it was not possible to capture the saturation of τ_m and τ_h to constant values for depolarized membrane potentials. This poor fit had catastrophic consequences, as illustrated in Fig. 4A. Due to the near-zero time constants at depolarized levels, the current activated and inactivated too fast and led to peak current amplitudes that were over an order of magnitude smaller than the Hodgkin-Huxley model with same channel densities (compare Fig. 4A with Fig. 1B). We conclude that linear thermodynamic models do not provide an acceptable behavior in voltage-clamp for the T-current.

Nonlinear Thermodynamic Models In general, the effect of the electrical field on a protein will depend on the number and position of its charged amino acids, which will result in both linear and nonlinear components in the free energy. Without assumptions about the underlying molecular structure, the free energy of a given state i can be written as a Taylor series expansion of the form

$$G_i(V) = A_i + B_i V + C_i V^2 + \ldots, \quad (23)$$

where A_i corresponds to the free energy that is independent of the electrical field, and the linear term $B_i V$

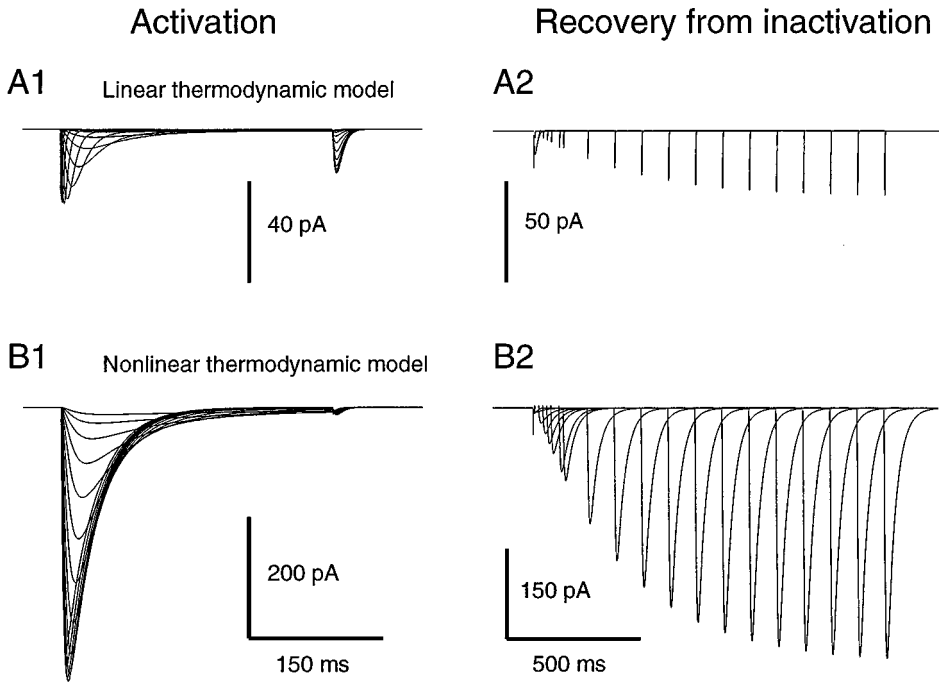

Figure 4. Voltage-clamp behavior of thermodynamic models of the T-current. *Left panels*: Activation protocol. *Right panels*: Protocol for the recovery from inactivation (same protocol as in Fig. 1). **A:** Linear thermodynamic model of the T-current. **B:** Nonlinear thermodynamic model. The same density of T-channels was used in all cases and was the same as the empirical models shown in Fig. 1B ($\bar{P}_{Ca} = 3 \times 10^{-6}$ cm/s).

corresponds to the interaction between electrical field with isolated charges and rigid dipoles, as described above. The higher-order terms describe effects such as electronic polarization and pressure induced by V (Hill and Chen, 1972; Stevens, 1978; Andersen and Koeppe, 1992), as well as mechanical constraints (see below).

In general, each conformational state of the ion channel protein will be associated with a given distribution of charges and will therefore be characterized by a given set of coefficients in Eq. (23). This is also true for the activated state, which is a particular case of conformation. Applying Eqs. (12) and (23), the rate constant can be written more generally as

$$\begin{aligned} r(V) &= r_0\, e^{-[(A^*+B^*V+C^*V^2+\ldots)-(A_0+B_0V+C_0V^2+\ldots)]/RT}, \\ &= r_0\, e^{-(a+bV+cV^2+\ldots)/RT}, \end{aligned} \tag{24}$$

where $a = A^* - A_0$, $b = B^* - B_0$, $c = C^* - C_0, \ldots$, represent differences between the linear and nonlinear components of the free energy of the initial and activated states (according to Eq. (23)).

Applying these considerations to the reversible open-closed transition (Eq. (16)) leads to the following general expression for the voltage-dependence of rate constants:

$$\begin{aligned} \alpha(V) &= \alpha_0\, e^{-(a_1+b_1V+c_1V^2+\ldots)/RT} \\ \beta(V) &= \beta_0\, e^{-(a_2+b_2V+c_2V^2+\ldots)/RT}, \end{aligned} \tag{25}$$

where a_1, a_2, b_1, b_2, c_1, $c_2, \ldots$, are constants specific of this transition.

It is important to note that, in general, these parameters are not necessary interrelated because the three different conformations implicated here (initial, activated, final as in Fig. 3) may have very different distributions of charges, resulting in different coefficients in Eq. (23) and thus also resulting in different values for $a_1, \ldots, c_2$. In the following, this general functional form for the voltage dependence of rate constants will be called *nonlinear thermodynamic model*.

In the low field limit (during relatively small transmembrane voltages), the contribution of the higher-order terms may be negligible, and one can approximate the free energy in various ways. The simplest approximation would only consider first-order terms in V, which gives the linear thermodynamic model (Eq. (17)). Other approximations would consist in quadratic or higher-order expansions of the free energy. These approximations include nonlinear effects of the voltage on the free energy, and may lead to saturating rate constants, as we will show below.

For example, the quadratic expansion of Eq. (25) can be written as

$$\begin{aligned} \alpha(V) &= A\, e^{-[b_1(V-V_H)+c_1(V-V_H)^2]/RT} \\ \beta(V) &= A\, e^{[b_2(V-V_H)+c_2(V-V_H)^2]/RT}, \end{aligned} \tag{26}$$

and similarly, its cubic expansion:

$$\begin{aligned} \alpha(V) &= A\, e^{-[b_1(V-V_H)+c_1(V-V_H)^2+d_1(V-V_H)^3]/RT} \\ \beta(V) &= A\, e^{[b_2(V-V_H)+c_2(V-V_H)^2+d_2(V-V_H)^3]/RT}, \end{aligned} \tag{27}$$

where $A, b_1, \ldots, d_2$ are constants as defined above.

In addition to the effect of voltage on isolated charges or rigid dipoles, described in Eq. (17), these forms account for more sophisticated effects such as electronic polarization or the deformation of the protein by the electrical field (Hill and Chen, 1972; Stevens, 1978). This contribution, however, was estimated to be small (see Sigworth, 1993). Higher-order terms would also take into account the presence of mechanical constraints on the gating process, as illustrated in Fig. 5. If gating results from the movement of a freely moving charge (Fig. 5A), the free energy will depend linearly on voltage. However, if gating depends on the movement of a gating charge subject to a mechanical constraint (modeled here as a spring of constant k; Fig. 5B), the force due to this constraint will contribute for a nonlinear term in the free energy ($-kV^2$ in the case of a spring). This model is probably more realistic than using a freely moving charge because charged residues in general are strongly constrained by the structure of the protein and are therefore not moving freely. Adding more complex constraints will likely results in adding further nonlinear contributions to the free energy (such as the third-order terms in Eq. (27)).

Nonlinear Thermodynamic Model of the T-Current

Nonlinear thermodynamic models (Eq. (25)) of different complexity were considered to fit the voltage-clamp data of the T-current. The quadratic expansion still provided a poor fit of the time constants, although better than linear fits (not shown). Acceptable fits were obtained for a cubic expansion of the rate constants, given by

$$\begin{aligned} \alpha_m(V) &= A_m \exp -[b_{m1}(V - V_m) + c_{m1}(V - V_m)^2 \\ &\quad + d_{m1}(V - V_m)^3]/RT \\ \beta_m(V) &= A_m \exp -[b_{m2}(V - V_m) + c_{m2}(V - V_m)^2 \\ &\quad + d_{m2}(V - V_m)^3]/RT \end{aligned} \tag{28}$$

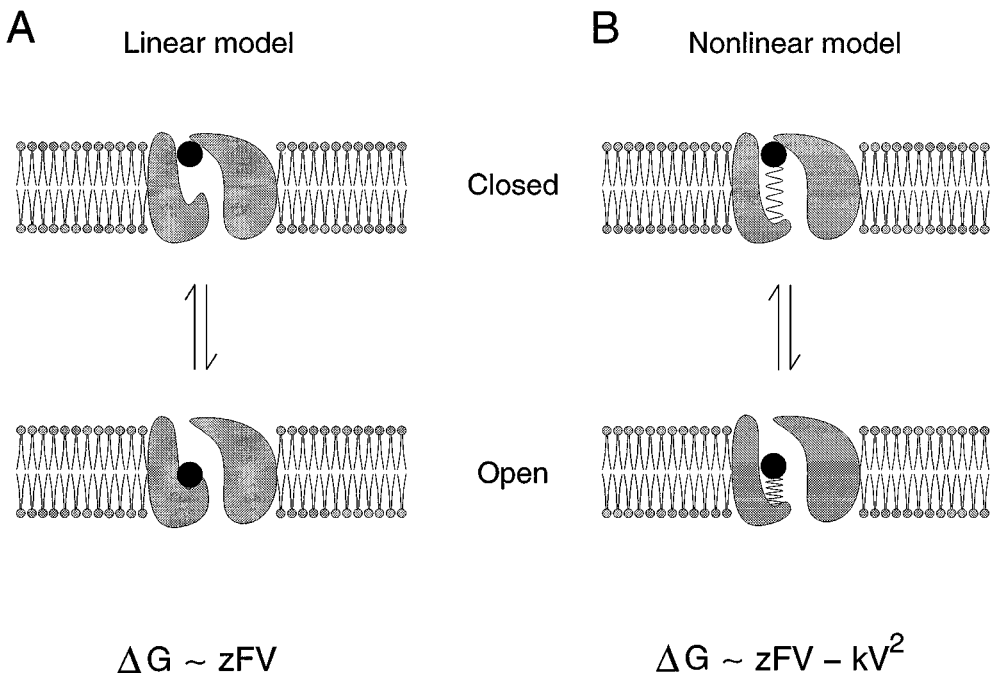

Figure 5. Models of ion-channel gating based on the movement of an electric charge inside the channel. **A:** A freely moving gating charge will result in a free energy that depends linearly on voltage. **B:** Imposing onstraints on the movement of the gating charge will add nonlinear terms in the free energy (see text). The example shown here illustrates the case of a gating charge attached to a spring of constant k, which will result in a quadratic voltage-dependence of the free energy.

$$\alpha_h(V) = A_h \exp -[b_{h1}(V - V_h) + c_{h1}(V - V_h)^2 + d_{h1}(V - V_h)^3]/RT$$
$$\beta_h(V) = A_h \exp -[b_{h2}(V - V_h) + c_{h2}(V - V_h)^2 + d_{h2}(V - V_h)^3]/RT.$$

The best fit of this nonlinear thermodynamic model is shown in Fig. 2 (thick dashed lines) and was obtained with the following parameters: $A_m = 0.053\,\text{ms}^{-1}$, $V_m = -56$ mV, $b_{m1} = -260$ J mV^{-1}, $c_{m1} = 2.20$ J mV^{-2}, $d_{m1} = 0.0052$ J mV^{-3}, $b_{m2} = 64.85$ J mV^{-1}, $c_{m2} = 2.02\,\text{J mV}^{-2}$, $d_{m2} = 0.036\,\text{J mV}^{-3}$, $A_h = 0.0017\,\text{ms}^{-1}$, $V_h = -80$ mV, $b_{h1} = 163\,\text{J mV}^{-1}$, $c_{h1} = 4.96\,\text{J mV}^{-2}$, $d_{h1} = 0.062\,\text{J mV}^{-3}$, $b_{h2} = -438\,\text{J mV}^{-1}$, $c_{h2} = 8.73\,\text{J mV}^{-2}$, $d_{h2} = -0.057\,\text{J mV}^{-3}$. Figure 2 (thick dashed lines) shows that this model could capture the form of the voltage dependence of the time constants. In particular, it could fit the saturating values for the time constants at depolarized levels. Nonlinear expansions of higher order provided better fits, but the difference was not qualitative (not shown).

Using these rate constants to simulate the voltage-clamp experiments on the T-current produced acceptable behavior, as shown in Fig. 4B. All protocols of activation (Fig. 4B1), deactivation (not shown), inactivation (not shown), and recovery from inactivation (Fig. 4B2) showed a similar voltage dependent behavior as the experimental data.

Genesis of Low-Threshold Calcium Spikes Finally, the different models shown above for the T-current were compared in current clamp. A single compartment model of the thalamic cell was generated (see Eq. (10) in Section, Methods) and contained leak currents and the T-current using one of the models described above. The genesis of the low-threshold calcium spike was monitored through return to rest after injecting hyperpolarizing currents. The Hodgkin-Huxley type model of the T-current generated low-threshold spikes in a grossly all-or-none fashion (Fig. 6A). The linear thermodynamic model (Fig. 6B) did not generate low-threshold spikes, consistent with the very small amplitude of the current evidenced above (Fig. 4A). On the other hand, the nonlinear thermodynamic model of the T-current presented a behavior more consistent with the Hodgkin-Huxley type model (Fig. 6C).

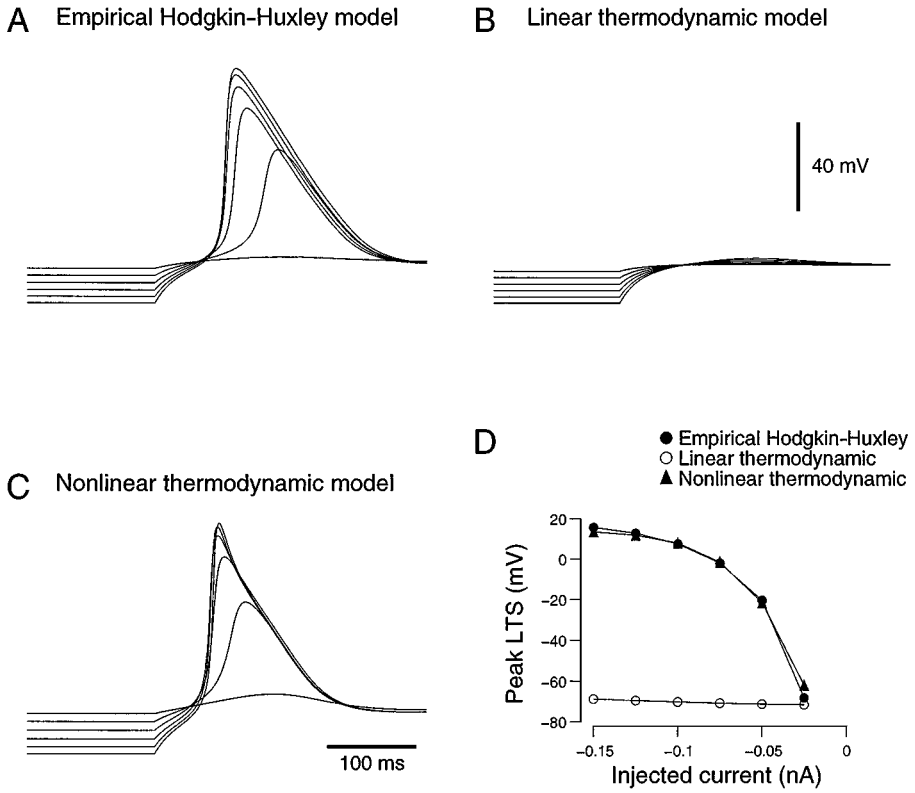

Figure 6. Low-threshold spikes generated by three different models of the T-current. Comparison of the same current-clamp simulation for three different Hodgkin-Huxley type models for the T-current: an empirical model (**A**), a linear thermodynamic model (**B**) and a nonlinear thermodynamic model (**C**). The simulation consisted in injecting hyperpolarizing current pulses of various amplitudes (-0.025, -0.05, -0.075, -0.1, -0.125, and -0.15 nA) and of 1 sec duration. At the end of the pulse, the model generated a low-threshold spike on return to rest. **D:** Peak amplitude of low-threshold spikes (LTS) generated by the different models of the T-current. All simulations were done using the same single-compartment geometry that constained leak currents in addition to the T-current (see Section 2, Methods). The density of T-channels was identical in all cases ($\bar{P}_{Ca} = 5 \times 10^{-5}$ cm/s) and was in the range of densities estimated from rat ventrobasal thalamic neurons (Destexhe et al., 1998).

Although the shape of the low-threshold spikes were not identical, their peak amplitudes were remarkably similar (Fig. 6D). We therefore conclude that nonlinear thermodynamic models provide fits of comparable quality to empirical Hodgkin-Huxley type models, using a functional form of the voltage-dependence derived from physically plausible arguments.

4. Discussion

In this article, we have shown that (1) Hodgkin-Huxley type models in which the transition rates are deduced from a linear dependence of the free energy on voltage fail to account for the voltage-dependent properties of some currents, such as the T-current in thalamic neurons, and (2) models in which the free energy depends nonlinearly on voltage can capture these properties. We discuss below possible applications of these nonlinear thermodynamic models.

4.1. Applications to Hodgkin-Huxley Models

As we have shown for the T-type calcium current, an important caveat of linear thermodynamic models is that the time constants may be arbitrarily close to zero. This is due to the fact that rate constants described by simple exponentials of voltage (Eq. (17)) can reach arbitrarily large values.[2] Different solutions to this problem have been proposed (Borg-Graham, 1991; Willms et al., 1999) and consisted in artificial alterations of the Hodgkin-Huxley formalism by either setting minimal

values for the rate constants or forcing the rate constants to saturate at extreme voltages. However, these procedures are largely empirical, which contrasts with the initial aim of thermodynamic models.

In reality, the rate of conformational changes of proteins are necessarily bounded by mechanical constraints and consequently, the transition time constants have a minimum value (e.g, Fig. 2C–D, symbols). We have explored here the hypothesis that this minimum value is due to nonlinear effects of the voltage on the ion channel. We have examined functional forms for the voltage dependence of rate constants that include effects such as deformation of the protein by the electric field (Hill and Chen, 1972; Stevens, 1978; Andersen and Koeppe, 1992) or mechanical constraints on the gating process (Fig. 5). These considerations lead to a more general form of the voltage dependence of the free energy. The resulting model can capture the saturation of the rate constants at some voltages and naturally leads to time constants saturating to a minimal value.[3] This type of model can therefore capture experimental data showing such a minimal time constant, as we have illustrated for the T-type current (Fig. 2C–D, symbols).

It must, however, be kept in mind that models in which the free energy is represented by a quadratic or third-order polynomial of V are approximations. In its nonapproximated form (Eq. 23), the free energy can be expressed as an infinite-order polynomial of V (Stevens, 1978). Approximating this form by a second- or third-order polynomial relies on the hypothesis that the voltage is not too large (the "low-field limit"). Consequently, these forms are adequate only for describing the behavior of the channel in a specific range of membrane potential. The example shown here for the T-current suggests that this range is large enough to apply to the usual range of membrane potential used in macroscopic voltage-clamp experiments. Outside this range, such as for very large voltages, the values of rate constants may diverge, and one must consider more complex expansions of the free energy, taking into account more sophisticated effects of the voltage on the channel structure.

Although nonlinear thermodynamic models capture the kinetics and voltage dependence of currents with an accuracy comparable to Hodgkin-Huxley models based on empirical functions, they may not be correct because the Hodgkin-Huxley formalism itself may be inadequate. It is possible that activation and inactivation are coupled, similarly to Na^+ channels (Armstrong, 1981; Aldrich et al., 1983; Bezanilla, 1985). In this case, the transitions leading to activation and inactivation should rather be modeled using Markov kinetic representations, as was done for the T-current in fibroblasts (Chen and Hess, 1990). Single-channel recordings should be used to decide which representation is most appropriate. In the case of the thalamic T-type current, such data are not yet available. At present, therefore, nonlinear thermodynamic models constitute an acceptable biophysical representation for this current, which is consistent with macroscopic voltage-clamp measurements in thalamic neurons.

4.2. Applications to Markov Models

The nonlinear thermodynamic formalism is not specifically related to the Hodgkin-Huxley model but is also applicable to any type of voltage-dependent model, including Markov representations. For example, when macroscopic voltage-clamp data reveal that the time constants saturate to a minimum value for activation, it is usually assumed (e.g., Chen and Hess, 1990) that this behavior requires two successive transitions:

$$C_1 \underset{\beta(V)}{\overset{\alpha(V)}{\rightleftarrows}} C_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} O, \tag{29}$$

where C_1 and C_2 are two distinct closed states of the gate, and the rate constants α and β are simple exponentials of voltage (Eq. (17)). Because the second transition is not dependent of voltage, it will act as a rate-limiting factor when α and β reach high values. In this case, the system will be governed essentially by k_1 and k_2, which impose a limit on the rate of activation and inactivation.

Although this scheme may be more realistic, it is still unrealistic that the simple exponential representation for α and β permits the first transition to occur arbitrarily fast at some voltages. This problem can be solved by using nonlinear thermodynamic models for α and β, leading to a simpler scheme with only one transition:

$$C_1 \underset{\beta(V)}{\overset{\alpha(V)}{\rightleftarrows}} O. \tag{30}$$

Here, the rate constants α and β are exponentials with an argument depending nonlinearly on voltage (Eq. (25)). Thus, in this case, the minimum value of the time constant is not interpreted as due to the existence of a

rate-limiting transition but as due to mechanical constraints in the effect of the electrical field on the protein. Nonlinear thermodynamic models therefore open the possibility of describing the gating of ion channels using more compact models with few states, which may be of great value for network simulations (Destexhe et al., 1994).

Acknowledgments

Research supported by grants from the Medical Research Council of Canada and the National Institutes of Health.

Notes

1. The term thermodynamics is used here to describe nonequilibrium phenomena (e.g., de Groot and Mazur, 1962; Kreuzer, 1981), which typically apply to ion channels during voltage-dependent transitions.
2. Very fast transition rates are however common in single channels. For a recent modeling of this aspect at the single-channel level, see Sigg et al. (1999).
3. In addition, to be more realistic, models with a minimal time constant are also more stable numerically.

References

Aldrich RW, Corey DP, Stevens CF (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature* 306:436–441.

Andersen O, Koeppe RE II (1992) Molecular determinants of channel function. *Physiol. Rev*. 72:S89–S158.

Armstrong CM (1981) Sodium channels and gating currents. *Physiol. Rev*. 62:644–683.

Bezanilla F (1985) Gating of sodium and potassium channels. *J. Membr. Biol*. 88:97–111.

Borg-Graham LJ (1991) Modeling the nonlinear conductances of excitable membranes. In: H Wheal, J Chad, eds. *Cellular and Molecular Neurobiology: A Practical Approach*. Oxford University Press, New York. pp. 247–275.

Chen C, Hess P (1990) Mechanisms of gating of T-type calcium channels. *J. Gen. Physiol*. 96:603–630.

de Groot SR, Mazur P. (1962) *Non-equilibrium Thermodynamics*. North Holland, Amsterdam.

Destexhe A, Mainen ZF, Sejnowski TJ (1994) Synthesis of models for excitable membranes, synaptic transmission and neuromodulation using a common kinetic formalism. *J. Comput. Neurosci*. 1:195–230.

Destexhe A, Neubig M, Ulrich D, Huguenard JR (1998) Dendritic low-threshold calcium currents in thalamic relay cells. *J. Neurosci*. 18:3574–3588.

Destexhe A, Sejnowski TJ (1997) Synchronized oscillations in thalamic networks: Insights from modeling studies. In: Steriade M, Jones EG, McCormick DA, eds. *Thalamus*. Elsevier, Amsterdam. pp. 331–371.

Eyring H (1935) The activated complex in chemical reactions. *J. Chem. Phys*. 3:107–115.

Eyring H, Lumry R, Woodbury JW (1949) Some applications of modern rate theory to physiological systems. *Record Chem. Prog*. 10:100–114.

Hill TL, Chen YD (1972) On the theory of ion transport across nerve membranes. VI. Free energy and activation free energies of conformational change. *Proc. Natl. Acad. Sci. USA* 69:1723–1726.

Hille B (1992) *Ionic Channels of Excitable Membranes*. Sinauer Associates, Sunderland, MA.

Hines ML, Carnevale NT (1997) The NEURON simulation environment. *Neural Computation* 9:1179–1209.

Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol*. 117:500–544.

Huguenard JR (1996) Low-threshold calcium currents in central nervous system neurons. *Ann. Revi. Physiol*. 58:329–348.

Huguenard JR, McCormick DA (1992) Simulation of the currents involved in rhythmic oscillations in thalamic relay neurons. *J. Neurophysiol*. 68:1373–1383.

Huguenard JR, Prince DA (1992) A novel T-type current underlies prolonged calcium-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *J. Neurosci*. 12:3804–3817.

Jahnsen H, Llinás RR (1984) Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurons *in vitro*. *J. Physiol*. 349:227–247.

Johnson FH, Eyring H, Stover BJ (1974) *The Theory of Rate Processes in Biology and Medicine*. J. Wiley, New York.

Johnston D, Wu SM (1995) *Foundations of Cellular Neurophysiology*. MIT Press, Cambridge, MA.

Kreuzer HJ (1981) *Nonequilibrium Thermodynamics and Its Statistical Foundations*. Oxford University Press, Oxford.

McCormick DA, Huguenard JR (1992) A model of the electrophysiological properties of thalamocortical relay neurons. *J. Neurophysiol*. 68:1384–1400.

Press WH, Flannery BP, Teukolsky SA, Vetterling WT (1986) *Numerical Recipes: The Art of Scientific Computing*. Cambridge University Press, Cambridge.

Sigg D, Quian H, Bezanilla F (1999) Kramers' diffusion theory applied to the gating kinetics of voltage-dependent ion channels. *Biophys. J*. 76:782–803.

Sigworth FJ (1993) Voltage gating of ion channels. *Quart. Rev. Biophys*. 27:1–40.

Stevens CF (1978) Interactions between intrinsic membrane protein and electric field. *Biophys. J*. 22:295–306.

Tsien RW, Noble D (1969) A transition state theory approach to the kinetics of conductances in excitable membranes. *J. Membr. Biol*. 1:248–273.

Willms AR, Baro DJ, Harris-Warrick RM, Guckenheimer J. (1999) An improved parameter estimation method for Hodgkin-Huxley models. *J. Comput. Neurosci*. 6:145–168.